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THE METABOLIC EFFECTS OF PANCREATIC HYPERGLYCEMIC-GLYCOGENOLYTIC FACTOR (GLUCAGON)‡

HISTORY

The presence of hyperglycemia-producing properties in pancreatic extracts and commercial insulin preparations was noted as early as 1923.[19, 86, 74] However, Fisher[86] believed the hyperglycemic activity resided in a "toxic fraction" which was also responsible for the local irritation and sterile abscesses associated with insulin therapy; and Collip's extracts[19] produced an unusually prolonged hyperglycemia of one to four days' duration. It is therefore uncertain whether the hyperglycemic effect of these early extracts was due to the specific hyperglycemic-glycogenolytic factor (HGF). In 1924, Kimball and Murlin[64] gave the name "glucagon" to the factor, but the earliest intensive investigators of its properties were the German workers, Bürger and Kramer,[8–11] who were dealing with the specific material and who demonstrated that the hyperglycemic effect was due to direct glycogenolytic effect on the liver.

After the first successful crystallization of insulin by Abel *et al.*[1] in 1927, Geiling and de Lawder,[40] and Bürger and Kramer,[11] demonstrated that crystalline insulin, even when injected intravenously, did not cause hyperglycemia. Glucagon was therefore considered merely an unimportant impurity, and during the next few years only Bürger and his colleagues in Germany continued its study. These workers were the first to attempt its purification.[8] In 1934 Scott[90] succeeded in crystallizing insulin by a new, highly efficient method which was widely adopted by most commercial manufacturers. Insulin prepared by this method was found to have hyperglycemic and hepatic glycogenolytic properties.[24] Lundsgaard *et al.* in 1939[71]

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noted that a preparation of insulin provided by Scott stimulated the release of glucose by the perfused liver, while a preparation of Abel's did not. De Duve *et al.*[24] demonstrated by means of constant intravenous glucose infusions to insulinized rabbits that Lilly insulin (prepared by Scott's method) was contaminated by the hyperglycemic factor, while a Danish preparation ("Novo") made by the Abel technique was not. Olsen and Klein[76] confirmed the hyperglycemic properties of most insulin preparations when given intravenously. "Novo" insulin was again an exception. This and other work[23, 25, 58, 83, 84, 89, 96, 100] presented strong evidence that insulin, per se, has no direct glycogenolytic effect on the liver and resulted in a shift of interest towards glucagon as an independent entity.

SITE OF ORIGIN AND DISTRIBUTION OF GLUCAGON

Sutherland and de Duve were able to extract the factor from the pancreas of several species, including man.[95, 98] They suggested that the alpha cells of the islets of Langerhans were the probable source because there were increased amounts per unit weight in the fetal calf pancreas, in the tail of the adult dog pancreas (where islets are concentrated), and in the duct-ligated and alloxan-diabetic pancreas in which acinar and beta cells have been destroyed. Pincus[78] confirmed these findings in the dog. Audy and Kerly[4, 5] noted a similar content of glucagon in pancreatic extracts from the rabbit, rat, cat, guinea pig, and ferret and determined that in the Lophius piscatorius, a teleost fish in which islet cell tissue is found outside the pancreas, extracts from islet cell tissue had a high content of glucagon, while acinar tissue had minimal HGF activity. Sutherland and De Duve[98, 99] were also able to extract a factor, apparently identical with HGF, from the upper three-fourths of the gastric mucosa, and, in small quantities, from the duodenum and ileum of the dog, but not from other tissues. They suggested that the glucagon found in these sites may be formed by the argentophil cells of the gastro-intestinal mucosa, which have a similar distribution and which stain with silver as do alpha cells. Although the two groups of cells are superficially similar, comparative morphological studies of Fodden[46] have shown that they are not related physiologically.

The site of origin of HGF has also been studied by the use of specific toxins. If glucagon is made in the alpha cells, destruction of these cells should cause a decrease in the pancreatic HGF content and an increase in the unopposed hypoglycemic action of the beta-cell hormone, insulin. In 1951, Van Campenhout and Cornelis[104] reported selective destruction of alpha cells after the administration of cobaltous chloride; and Vuylsteke, Cornelis, and De Duve in 1952[108] reported a decreased HGF content of the

pancreas of animals pretreated with cobalt. Cavallero[15] reported that pancreatic extracts from fish previously treated with cobalt had no hyperglycemic effects after alkali inactivation of insulin. However, other work with cobalt has failed to confirm some of the above findings and does not support the concept of the alpha cell origin of the HGF. Goldner, Volk, and Lazarus have demonstrated that hyperglycemia following immediately after cobalt administration is not due to a predestructive "excitation initiale" of the alpha cells, as proposed by Van Campenhout and Cornelis,[104] but occurred regardless of the intervals between successive doses[107] and increased with increasing doses of cobalt[51]; indeed, the initial hyperglycemia occurred in the pancreatectomized animal.[97] The hepatic glycogen content of cobalt treated animals was decreased. They therefore ascribe the hyperglycemic response to a direct hepatic effect of cobaltous ions.[106] These authors also reported that following destruction of the alpha cells by cobalt, there was no hypoglycemia,[106] no amelioration of alloxan diabetes,[97] and a persistence of hyperglycemic activity in pancreatic extracts.[52] Raja Rama Rao and De,[82] working with rabbits, noted no decrease in HGF content of the pancreas after cobalt administration. These authors also claim to have detected HGF in the abdominal lymphatics and spleen as well as in the pancreas and gastric mucosa, and, on this basis, suggested abdominal lymphatic tissue as the source of HGF. However, they do not mention their method of extraction.

Deca methylene diguanidine hydrochloride, or Synthalin A, was shown by Davis,[22] in 1952, to produce a selective but inconstant hydropic change of the alpha cells in rabbits. These changes are not nearly as extensive as those caused by cobalt, but are followed by a hyperglycemia of several hours' duration, and then marked hypoglycemia. Fodden,[47, 48] comparing the effects of cobalt and Synthalin A, suggested that cobalt causes a retention of HGF in the alpha cells, while Synthalin A, after causing an initial secretory discharge of HGF, inhibits completely the HGF function of the cells. He believes that this explanation reconciles the incompatabilities between alpha cell destruction and glycemic effects of the two compounds, and that his work supports the alpha cell origin of pancreatic HGF.

In summary, there is abundant evidence of the presence of a factor producing hyperglycemia and hepatic glycogenolysis in the pancreas of all species that have been assayed for the substance. The bulk of evidence supports the alpha cells as the pancreatic cellular site of origin. The main objection to this concept is the inconsistent effect obtained with the specific cytotoxins. However, for several reasons, work with the cytotoxins cannot be evaluated completely at this time. Neither of the toxic compounds has been shown to destroy all of the alpha cells, and although cobalt in some

dosages appears to produce considerable alpha cell damage, its effects on other gastro-intestinal sources of HGF are unknown. Indeed, Fodden[46] noted no effect on the gastro-intestinal argentaffin cells after either cobalt or Synthalin A. Furthermore, these substances may not affect carbohydrate metabolism entirely by damaging alpha cells, since both probably have primary hepatic glycogenolytic activity.

PROPERTIES AND POTENCY

A detailed account of the methods of extraction and the physical and chemical properties of the pancreatic HGF is not within the scope of this paper. Suffice it to say that HGF is apparently a polypeptide physically somewhat similar to insulin but differing from the latter in its amino acid composition, and its resistance to alkali and cysteine incubation.[98] It has been demonstrated that the hyperglycemia obtained with cysteine-inactivated pancreatic and gastric extracts is due to HGF and not to the hyperglycemic effect of cysteine itself.[39] HGF has been extracted in a purified crystalline form by Staub *et al.*[98] It can be assayed *in vivo* by measuring the hyperglycemic effect, but its glycogenolytic effect on rabbit liver slices[96, 98, 99] is the most accurate method. The material is extremely potent in its purified form, 0.1 μg./Kg of body weight causing a significant hyperglycemia in the cat[98]; as Foá[37] points out, on a weight basis, the potency of HGF is at least ten times greater than that of insulin.

Carbohydrate metabolism. The best-established effect of HGF is the production of a rapid, transient hyperglycemia, accompanied by marked glycogenolysis in the liver.[96] The fact that this hyperglycemia is most marked after intraportal injection,[10, 18] that it is absent in the hepatectomized animal,[62, 80] is increased when liver glycogen content is high,[59, 91] and diminished in situations where liver glycogen is decreased—as in diabetic animals in ketosis,[38, 41, 115] in patients with cirrhosis,[75] and in fasting animals[110]—all attest to the primary hepatic site of action. Sutherland and Cori[95–97] have conclusively demonstrated that the glycogenolytic effect of HGF, like that of epinephrine, is on the liver phosphorylase system, both agents probably acting upon a liver enzyme that maintains a balance between active and inactive phosphorylase. However, despite their similarities with respect to hyperglycemic and hepatic glycogenolytic effects, HGF and epinephrine have numerous points of difference. Epinephrine causes an increase in blood lactate levels, a result of its glycogenolytic effect in muscle, and this lactate secondarily replenishes hepatic glycogen stores.[21] HGF does not cause increased blood lactate levels after administration to normal animals[95, 109] or normal or diabetic human beings.[65] Furthermore, HGF does

not have the pressor influence on blood pressure and pulse that epinephrine manifests.[109]

The fact that epinephrine has effects on muscle tissue slices[20] and in the hepatectomized animal[95] is confirmatory of its peripheral, extra-hepatic activity. There are few data to support such activity on the part of HGF. The most impressive evidence of an extra-hepatic action of glucagon is the work of Drury, Wick, and Sherrill[28] who maintained eviscerated, nephrectomized rabbits at constant blood sugar levels by accurately measured intravenous glucose injections. Some were given insulin simultaneously. On the basis of the amount of glucose required to maintain constant blood levels, these authors reported that glucose transfer was decreased during HGF administration and suggested that HGF may play a rôle as an insulin antagonist. HGF, like insulin, had no effect on the rate of glucose oxidation, however. Rodriguez-Candela[97] has reported that HGF inhibits the deposition of glucose in the isolated rat diaphragm in the presence of insulin. HGF alone, however, had no effect. Young[114] was unable to confirm these results; nor could Mirsky[72] demonstrate any effect of HGF on glycogen synthesis in the rat diaphragm. Pincus and Rutman,[81] working with normal dogs, reported that HGF results in an *increase* in muscle glycogen in the presence of insulin. Engel and Scott[31] have reported that HGF had no effect on glycogen synthesis in rat adipose tissue.

Lipid metabolism. Haugaard and Stadie,[58] and Haugaard and Haugaard[57] working with rat liver slices, found a decrease in fatty acid synthesis in the presence of HGF, as measured by the incorporation of radioactive labeled acetate, glucose, or fructose into fatty acids. These authors point out that the decreased specific activity of the fatty acids produced under their experimental conditions might be explained on the basis of a dilution of the pool of fatty acid precursors with glucose released by the glycogenolysis induced by HGF but they believe that the amount of glycogenolysis was inadequate to explain the effects they observed. Haugaard and Haugaard also noted an increased ketone body synthesis in liver slices treated with HGF. Foá and Weinstein,[43, 44] working with diabetic dogs, found an increased ketonemia after administration of a Lilly purified HGF, but using other crude pancreatic extracts, they noted a hyperglycemic effect with a marked *decrease* in blood ketones. These authors suggest that there is a pancreatic factor, distinct from insulin and HGF, which is capable of decreasing the blood ketone levels. Stewart and Roitman[94] obtained no effect on ketone production by rat liver slices after treatment with Lilly purified HGF or various aqueous pancreatic extracts. Zimmermann and Donovan[115] gave cysteine-inactivated insulin preparations as a source of

HGF to depancreatized dogs untreated with insulin and noted a brief increase followed by a prolonged diminution in ketonemia. These changes accompanied a similar brief hyperglycemia and prolonged hypoglycemia in the dogs. Kalant,[63] using fasted rats and intraperitoneal Lilly purified HGF, also noted a significant decrease in ketonemia acutely, and an inhibition of ketosis during fasting. It should be noted that this author reported no significant accompanying hyperglycemia nor depletion of liver glycogen, and although the absence of these phenomena can probably be explained by the fact that the animals were in the fasted state and that blood samples for analysis were apparently taken two hours after the last dose of HGF, the efficacy of HGF administered by the intraperitoneal route is open to question.[31, 80] Rutman, Pincus, Brown, and Scott,[88] found no changes in blood acetone levels of intact and pancreatectomized dogs after HGF administration. The blood lipids did not change in glucagon-treated dogs when compared to saline- or insulin-treated controls.[88] Pincus and Rutman[81] reported that HGF did ameliorate the acidosis of diabetic dogs. Bondy and Cardillo were unable to produce ketosis by intravenous glucagon in fasted normal human beings.[6]

The observed effects of HGF on fat metabolism have been contradictory. The pathways of lipid and carbohydrate metabolism are closely interrelated and converge to a common enzyme system at the level of acetyl-Co-A. The effect of glucagon on ketosis, therefore, may depend on its ability to alter the liver glycogen and the availability of glucose to the liver and peripheral tissues. The variability of the reported effects probably results from the variety of conditions under which the tests have been performed, and the consequent variations in the basic substrate on which the hormone could act. There is no clear-cut evidence that glucagon has any effect on fat metabolism except as a consequence of the primary alterations it produces in carbohydrate metabolism.

Protein metabolism. The rôle of the HGF in protein metabolism has not as yet been investigated. Tyberghein,[103] measuring urinary nitrogen excretion in rabbits, reported that HGF accelerates protein catabolism.

DIABETOGENIC ACTIVITY

The effect of pancreatectomy on experimental diabetes caused by insulin deficiency. If the pancreatic hyperglycemic factor is diabetogenic, it should be possible to alleviate established experimental diabetes caused by beta-cell destruction by removal of the remainder of the pancreas. Experiments of this type have been carried out by a number of authors. Young, in 1939,[113] reported that dogs made permanently diabetic by injections of anterior

pituitary extracts required more insulin than pancreatectomized dogs on a similar diet. Dragstedt, Allen, and Smith[27] noted that dogs who were 90% to 95% depancreatized required more insulin to maintain minimal glycosuria than a similar series of totally depancreatized animals. Cavallero and Malandra[14] reported that removal of 90% of the pancreas reduces the glycosuria and fasting blood sugar, and increased the insulin sensitivity of alloxan-diabetic rats. Thorogood and Zimmermann, in 1945,[102] reported that pancreatectomy in alloxan-diabetic dogs reduced the glycosuria and insulin requirements. These authors also noted that ketosis and coma developed more rapidly in the pancreatectomized dogs after insulin withdrawal and suggested that there might be an alpha cell hormone which acts in opposition to insulin in respect to blood sugar and glycosuria and which prevents ketosis and coma in the insulin-deficient animal.

Opposed to this explanation is the opinion of Mirsky.[78] This investigator confirmed the findings of Thorogood and Zimmermann[102] and of Rodriguez-Candela[86] that the quantity of glucose excreted in the urine by the alloxan-diabetic dog on a constant regimen of food and insulin is decreased after pancreatectomy, and that, therefore, the pancreatectomized animal will have lower insulin requirements if glycosuria is the criterion for control. However, this decreased glycosuria was not accompanied by a weight gain; after pancreatectomy of alloxan-diabetic animals, there was an increase in hyperglycemia, ketonemia, and glycosuria during periods of fasting and of insulin deprivation; despite administration of sufficient pancreatin to prevent fatty stools and fatty infiltration of the liver in the pancreatectomized dogs, such dogs absorbed considerably less protein than alloxan-diabetic dogs: the alloxan-diabetic dogs have appreciable stores of liver glycogen, since they developed a marked ketonemia only after the administration of phlorizin with consequent depletion of liver glycogen. On the basis of these findings, Mirsky concluded that the "amelioration" of alloxan diabetes by pancreatectomy is on the basis of decreased protein absorption with a resulting diminution of carbohydrate precursors, and that the resistance to ketosis and coma in the alloxan-diabetic animal before pancreatectomy is on the basis of the ability of these animals to store large quantities of glycogen. He would, therefore, ascribe the differences between alloxan-diabetic and pancreatectomized animals to the exocrine rather than to the alpha cell function of the pancreas. There are certain experiments which seem to contradict this theory. Thus, Thorogood and Zimmermann[102] and Rodriguez-Candela[86] noted no decrease in the insulin requirement of the alloxan-diabetic dog after ligation of the pancreatic duct, while the removal of the remaining sclerosed pancreas did result in a decreased insulin requirement. However, Thorogood and Zimmermann reported on only two dogs subjected to duct

ligation and Rodriguez-Candela offers no details as to the observations he reported. These experiments have also been criticized because the dogs were not observed long enough after ligation of the pancreatic duct to permit a defect of protein absorption to develop.

Cavallero and Malandra[12, 13, 15] reported that pancreatic grafts from alloxan-diabetic, but not from normal, rats reduced the glucose tolerance and increased the insulin resistance of rats made diabetic by partial pancreatectomy.

Total pancreatectomy in man. In recent years a limited number of totally depancreatized human beings have been available for observation. Although many of the patients die too soon postoperatively to permit adequate metabolic study, a few have been investigated in some detail months after surgery and in a more or less stable condition. Dixon *et al.*,[26] Ricketts *et al.*,[85] and Fallis and Szilagyi[38] have reported the results of total pancreatectomy in previously diabetic patients. Summaries of studies on depancreatized humans have been published by Whipple in 1946[112] and Gourevitch, Thomas, and Whitfield in 1952.[54] In general, these patients require less insulin than many ordinary diabetics, since their maintenance dosage usually falls in the range of twenty to forty units daily. The previously diabetic patients required about the same amount of insulin as preoperatively. However, these patients all suffer from defective nitrogen and fat absorption, the number adequately studied has been small, the criteria for regulation are not uniform, and individual complicating variations are numerous, so that little clarification of the rôle of HGF as a diabetogenic agent has come, as yet, from studies of pancreatectomized humans.

Experimental production of diabetes by HGF. Cavallero and Malandra[16] reported that HGF increased the blood sugar levels and glycosuria in rats force-fed a diet high in carbohydrate, and that it enhanced significantly the hyperglycemic and glycosuria effects of pituitary growth hormone, adrenocorticotropic hormone, and cortisone. Glucagon, while not diabetogenic itself, potentiates the diabetogenic effects of cortisone in respect to glycosuria, fasting blood sugar levels, and glucose and insulin tolerance tests in intact, *ad. lib.* fed rats.[17] However, cortisone diabetes is characterized by elevated hepatic glycogen stores and the demonstration that HGF, with its marked hepatic glycogenolytic action increases the glycosuria and basal blood sugar levels of these animals is not significant evidence of a true diabetogenic effect. Ingle, Beary, and Purmalis[61] noted that HGF produced a definite but temporary enhancement of glycosuria in partially depancreatized rats. This effect was produced only when HGF was administered by intravenous

infusion, and the glycosuria returned to pretreatment levels while HGF was still being administered.

The effects of HGF have been studied in humans in an effort to determine whether a state of hyperglycemia associated with decreased peripheral utilization of glucose could be produced. Van Itallie *et al.*[105] administered HGF by infusion to normal subjects in the postabsorptive state and reported that the HGF-induced hyperglycemia was accompanied by an increased difference in the levels of arterial and venous glucose. This finding indicates that there is no inhibition of peripheral glucose transfer under the influence of HGF as there is after epinephrine administration. Indeed, the work of these authors suggested that HGF might enhance glucose transfer on the basis of comparable determinations in subjects to whom glucose had been administered. Bondy and Cardillo[6] confirmed these findings in their series, and in addition noted that glucagon and glucose caused comparable decreases in blood inorganic phosphorus and alpha amino nitrogen levels. Kirtley *et al.*[65, 66] also produced a fall in serum inorganic phosphorus levels in normal subjects during and after HGF administration. A reduction of the levels of inorganic phosphorus appears to be a reliable manifestation of peripheral hexose transfer in normal subjects.[6]

There has been no report of permanent experimental diabetes resulting from HGF administration.

Increased alpha to beta cell ratio as the etiology of diabetes mellitus. Ferner[34, 35] suggests that the anatomical basis of human diabetes mellitus is an increased ratio of alpha to beta cells, with a resulting predominance of the "glucagon system" over the insulin system. He supports his theory with morphological studies of the islets of normal and diabetic humans[34, 35, 101] as well as the aforementioned investigations relative to the effect of total pancreatectomy on alloxan-diabetes, and by the well-known phenomenon of "amelioration' 'of pancreoprivic diabetes by hypophysectomy (with consequent removal of the alleged "alphacytotrophic principle" of the anterior pituitary). However, pancreatectomy removes the chief source of glucagon, and it is therefore most unlikely that any important part of the effect of hypophysectomy on the diabetes of pancreatectomized animals is a result of removal of the stimulus to the extra-pancreatic sources of HGF. The question of the "amelioration" of alloxan-diabetes by pancreatectomy has already been discussed. As Fabrykant[32] points out, different investigators are not in complete agreement as to the normal range of the alpha : beta ratio.[58] Furthermore, Ferner himself has demonstrated that there is an increased alpha : beta ratio in newborns, who ordinarily have low levels of blood glucose.[60]

Hyperglycemic substances in diabetic urine. One investigator[77] has reported the presence of a hyperglycemic-glycogenolytic factor in the urine of diabetic patients and alloxan-diabetic rats and rabbits, when the blood sugar levels were elevated above 200 mgm%. The action of this substance was destroyed by tryptic digestion and the author suggests that it is probably of pancreatic origin. Another report[111] of a hyperglycemic factor in diabetic urine suggests that the responsible substance is not a protein. Work in this line cannot be evaluated at this time.

HGF AS A MEDIATOR OF THE EFFECTS OF GROWTH HORMONE

It has been suggested that there is a relationship between an anterior pituitary "alphacytotrophic factor" closely related to growth hormone (STH) and the HGF, and that the HGF may mediate the diabetogenic effects of STH.[12, 85] The effects of purified growth hormone are reviewed by Young.[114] For the purposes of this discussion it can be pointed out that in animals these preparations have well-established growth accelerating properties which can be measured by epiphyseal cartilage growth or by the production of positive nitrogen balance, and that they have definite diabetogenic properties in some species. The evidence linking the growth hormone and HGF is as follows:

Histologic studies after hypophysectomy or administration of anterior pituitary extracts. Sonenberg[92] reported that isotopically-labeled growth hormone is concentrated in the pancreas. Ferner[85] found that hypophysectomy was associated with atrophy and a decrease in number of alpha cells, with no alterations in beta cells in all of the guinea pigs, and in one-half of the rats in his series, but in five or six months the cell pattern returned to normal. He also reported increased numbers of alpha cells as compared to the number of beta cells in human embryos and newborns and cited this finding as evidence associating the growth hormone, alphacytotrophic factor, and alpha cell system. Ham and Haist[55] treated dogs with daily injections of anterior pituitary extract and reported evidence of alpha cell degranulation in a small percentage of their animals. Cavallero[12] reported that rats pretreated with growth hormone preparations showed a marked increase in the ratio of mitotic activity of alpha cells to beta cells. Similar observations were made in dogs made diabetic by anterior pituitary extract injection. They also treated normal dogs with pancreatic extracts from these "Young diabetic" dogs and from normal animals and reported a more marked and prolonged hyperglycemia in the former animals.

Secretion of HGF in response to growth hormone. Bornstein, Reid, and Young[7] noted a hyperglycemic and glycogenolytic effect of portal or

pancreatico-duodenal venous blood but not of peripheral blood in intact and adrenalectomized hypophysectomized alloxan-diabetic animals treated with growth hormone. Growth hormone by direct injection and blood from saline-treated control animals failed to elicit the HGF effects in the animals used for assay. Foá *et al.*[40] reported that blood from the pancreatico-duodenal vein, but not from the mesenteric vein of donor dogs treated with growth hormone, caused a transient hyperglycemia in recipient dogs that was similar to the typical response to HGF. Blood from control animals injected with control solutions of the identical pH had no hyperglycemic effect.

It is important that growth hormone is capable of producing permanent diabetes in experimental animals,[114] but that HGF, even in high sustained dosages, is not. This does not support the concept that the diabetogenic effects of growth hormone are mediated entirely by HGF.

Somatotrophic effects of HGF. Elrick[29] treated hypophysectomized rats with HGF and reported that in most instances there was a significant increase in epiphyseal cartilage growth as compared to controls. He suggested that both the diabetogenic and growth-stimulating actions of growth hormone are mediated, in part or wholly, through the release of HGF. However, Geschwind and Staub,[50] using highly purified HGF preparations, were unable to confirm these results.

CURRENT STATUS

Is glucagon a hormone? It is a substance which exerts its effects on a distant organ and is potent in minute doses. It probably is formed chiefly in the alpha cells of the pancreas. Cavallero points out[12] that these cells have the characteristics of an independent endocrine organ, since they show morphological evidence of secretory activity, and their reactions to cytotoxins and physiological and pathological conditions are independent of the beta cells and the acini. The cross-circulation experiments of Foá and his collaborators[42, 46] have established the fact that the pancreas secretes a hyperglycemic substance into the blood stream in response to insulin-induced hypoglycemia. Work with perfusion of the isolated rat pancreas suggests the same conclusion.[2, 3] It has been impossible, however, to produce a pure and total deficiency of HGF. The rapid action and potency of glucagon and the evidence afforded by cross circulation experiments indicate that the HGF is probably one of the factors responsible for the regulation of blood glucose levels in the intact animal, possibly in response to anterior pituitary stimulation under some circumstances. Engel suggests[30] that HGF, like epinephrine, is involved in the early response to hypoglycemia. However,

the failure to obtain permanent hypoglycemia after destruction of most, if not all, of the alpha cells by cobalt, the demonstration that the response to hypoglycemia is not altered by pancreatectomy,[68, 69] the fact that liver glycogen is not diminished in alloxan-diabetic animals[73, 88] and the transient nature of HGF effects make it unlikely that the HGF plays a dominant rôle in metabolic homeostasis.

Glucagon is often referred to as an "anti-insulin" factor. This statement is true only in the most limited sense. It is true that, whereas insulin reduces the concentration of glucose in the blood, glucagon raises it; but these effects are produced in totally different sites and by unrelated mechanisms. Thus insulin appears to act chiefly on the peripheral tissues by increasing the entry of glucose into the cellular metabolism,[56, 70] whereas glucagon acts chiefly by promoting the breakdown of liver glycogen and its release as glucose into the blood. It is therefore clear that glucagon and insulin act synerigistically, the former by making liver glycogen stores available for the peripheral action of the latter. In insulin-deficient animals[12, 37, 38, 41] and human beings before hepatic glycogen is depleted[66] the hyperglycemic effects of HGF are exaggerated, but this is doubtless because under these circumstances the second link in the chain—insulin—is absent.

There is, therefore, no convincing evidence of any peripheral effect of HGF; indeed, at this time, the only effects that can be considered established are its hyperglycemic and hepatic glycogenolytic properties, which were demonstrated by Bürger and Kramer more than thirty years ago.

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